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Spawning Success of Fathead Minnows on Selected Artificial Substrates

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ABSTRACT: Spawning success of fathead minnows (*Pimephales promelas*) on six different substrates was tested and evaluated. Egg adhesiveness was equally good on cement-asbestos tile and sand-coated stainless steel substrates, but was poor on unaltered stainless steel, shot-peened stainless steel, stainless steel screen, and glass substrates. Viability and number of eggs spawned were not significantly different for any of the substrates tested.

The life history of the fathead minnow (*Pimephales promelas*) was described by Markus (1934) and Hester et al. (1946). They concluded that these fish laid their adhesive eggs on the undersurface of objects such as rocks, boards, concrete, tile, or sheet metal. Spawning substrates described by Mount and Stephen (1967) consisted of cement-asbestos drain tiles that were cut into sections and then halved lengthwise and inverted to form a semicircular arch. Nebeker et al. (1974) described similarly shaped spawning substrates made from brown glass beverage bottles. Another substrate recently developed by C. E. Stephen of this laboratory (personal communication) consisted of a plain sheet of stainless steel. The glass and stainless steel substrates were less porous than cement-asbestos tiles and could easily be cleaned and used again after each test, especially with organic toxicants. Although fathead minnows spawned readily on these materials, no attempt was ever made to evaluate or compare the spawning success on these substrates.

The purpose of this study was to determine the viability and adhesiveness of fathead minnow eggs spawned on cement-asbestos, glass, and several types of stainless steel substrates.

Methods

Six spawning substrates were constructed and tested. Substrate I consisted of inverted cement-asbestos half tiles 10 cm in diameter and 10 cm long. Substrates II, III, IV, and V were constructed from sheets of stainless steel bent in the same shape and size as substrate I. For substrate II a layer of silicone adhesive (0.25–0.50 mm thick) was spread evenly on the undersurface of the steel and 0.5- to 1.5-mm quartz sand (sifted through a screen with 1.5-mm openings to remove larger particles and

other debris) was sprinkled over the glue. Excess sand was shaken off and the remaining particles pressed down firmly with the finger. Inspection of the substrate surface showed that the silicone adhesive did not squeeze up between the particles, and that more than 90% of the silicone was covered with sand. The underside of substrate III was covered with a 40-mesh stainless steel screen made of 0.01-mm wire with 0.5-mm openings. The screen was held in place by arching it evenly against the underside of the metal and bending the excess screen tightly around the outside bottom edges. The underside of substrate IV (designed by C. E. Stephen) was textured by shot-peening with #220 aluminum oxide grit at 80 psi for 1 min. Substrate V consisted of a sheet of bent stainless steel with no alterations to the underside. Substrate VI was constructed from 9-cm diameter brown glass bottles that were cut in 10-cm sections. The sections were halved lengthwise to form the substrate. All substrates were thoroughly washed and rinsed before testing.

The tests were conducted in glass spawning tanks, 61 x 31 x 31 cm, containing water 20 cm deep. Flow rates averaged 100 ml/min, and temperatures varied from 24 to 25°C. All tests were conducted with a constant 16-h photoperiod. Traps were placed under each substrate to catch the eggs that did not adhere to the substrate. Each egg trap consisted of a shallow glass box 13.0 x 13.0 x 0.8 cm and a stainless steel screen with 5-mm openings suspended 3 mm above the bottom to prevent fish from reaching and eating the eggs. All traps were cleaned daily. Five substrates of each type were tested in four separate spawning tanks for about 1 week. The substrates were then removed, and the next set of substrates was tested with the same group of fish. Substrate I was tested first; the remaining substrates were tested in the order V, III, II, VI, and IV. Four cement-asbestos

substrates without traps were also placed in each spawning tank during the test to reduce territorial fighting among males.

We used mature 1-yr-old fathead minnows from the culture unit maintained at our laboratory. Three males and six females were placed in each of the four spawning tanks. Mean length and weight of male spawners were 8.8 cm and 3.8 g, and of females 5.9 cm and 1.6 g. Spawnings were checked daily, and eggs on the substrate and in the trap were removed by the finger pressure method (Gast and Brungs 1978) and counted. Ruptured eggs were also counted after removal from substrates I, II, and IV. Eggs adhering to the other substrate types came off so easily there was no need to count ruptured eggs. Subsamples of 50 eggs spawned on each substrate were incubated for 24 h to determine viability.

Results and Discussion

All test substrates placed in the territory of dominant males were readily accepted, and spawning generally began by the following day. Another indication of acceptance was the small difference in mean total eggs per spawning: the maximum difference between all test substrates was only 55 eggs (Table 1). The percentage of eggs found on substrates III, IV, V, and VI was significantly less than that on substrate I ($P < 0.01$, analysis of variance and Student's *t*-test); however, on substrate II the percentage of eggs adhering to the undersurface was not significantly different from the percentage on the cement-asbestos substrate (Table 1). Nearly 50% of the spawnings on substrates V and VI would have been missed if the egg traps had not been

present. This number was reduced to 7% for substrates III and IV and to zero for substrates I and II. Observations of several spawnings on substrates IV and V revealed that most of the eggs found in the trap fell off either during spawning or shortly afterward. All eggs that adhered to substrate IV were extremely difficult to remove, and much time and patience were needed to roll them off with as little pressure as possible. In spite of these extra precautions, a count of ruptured eggs during removal from the substrate showed a significant increase over substrates I and II ($P = 0.01$, Table 1). Viability of eggs was not significantly different for any of the substrates tested ($P = 0.01$), and viability of all eggs found in the traps was nearly the same.

Differences in spawning success of fathead minnows using different substrates are the result, therefore, of differences in the adherence of eggs to the material and not of differences in viability or in the number of eggs spawned. Our tests showed that egg adhesiveness was equally good on cement-asbestos tile and sand-coated stainless steel substrates, but was poor on unaltered stainless steel, shot-peened stainless steel, stainless steel screen, and glass substrates. These results demonstrate the importance of evaluating laboratory spawning substrates before using them in a life-cycle chronic exposure.

(Note: Incidental to the research reported here, we developed a simple technique to remove the silicone-sand coating from the stainless steel. Substrates were soaked for 24 h in hexane, after which the layer was easily peeled off with tweezers. A new coating could then be reapplied to the stainless steel in 5-8 min. In tests with certain toxicants, disposal of the used spawning surface would eliminate all toxicants accumulated on the substrate.)

Table 1. Spawning success (mean $\pm$ 95% confidence interval) of fathead minnows on various substrates (number of spawnings checked shown in parentheses).

Substrate	Mean number of eggs per spawn (substrate + trap)	Mean percentage of eggs adhering to substrate	Mean percentage of viable eggs	Mean percentage of eggs ruptured during removal from substrate
I. Cement-asbestos tile	118 $\pm$ 72 (12)	98 $\pm$ 4 (12)	78 $\pm$ 14 (8)	4 $\pm$ 2 (12)
II. Sand-coated stainless steel	122 $\pm$ 61 (14)	88 $\pm$ 3 (14)	88 $\pm$ 7 (8)	4 $\pm$ 3 (12)
III. Stainless steel screen	108 $\pm$ 42 (14)	57 $\pm$ 20 ^a (14)	78 $\pm$ 12 (12)	b
IV. Shot-peened stainless steel	127 $\pm$ 97 (12)	57 $\pm$ 19 ^a (12)	82 $\pm$ 6 (10)	17 $\pm$ 6 ^a (12)
V. Stainless steel	122 $\pm$ 42 (11)	17 $\pm$ 21 ^a (11)	80 $\pm$ 11 (5)	b
VI. Brown glass	108 $\pm$ 39 (12)	17 $\pm$ 12 ^a (12)	79 $\pm$ 10 (11)	b

^a Significantly different from substrates I and II, $P < 0.01$ (analysis of variance and Student's *t*-test).

^b Not counted.

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